Principal regulatory programs of amino acid metabolism in plants

Department of
Plant Sciences

Prof. Gad Galili

Hanna Levanony,
Shai Ufaz,
Hadar Less,
Ruthie Angelovici,
Vered Tzin,
Tamar Avin-Wittenberg

☎ 972 8 934 3511
FAX 972 8 934 4181
@ gad.galili@weizmann.ac.il
www.weizmann.ac.il/Plant_Sciences/galili/

Because amino acids are incorporated into proteins, they have traditionally been thought to represent end products of metabolic pathways. Moreover, since both in prokaryotes and eukaryotes the biosynthesis of amino acids is generally regulated by feedback inhibition loops of allosteric biosynthetic enzymes by the amino acids themselves, these feedback inhibition loops have been considered as the central regulators of amino acid metabolism. Yet, the notion that amino acids are end products of metabolic pathways is incorrect since amino acids are intermediates in metabolic pathways and are catabolized to various products having multiple functions. We are studying the regulation and functional significance of metabolic pathways of amino acids in plants, using genetic, metabolomic and bioinformatic approaches. Our genetic approaches use: (i) genetically engineered allosteric biosynthetic enzymes whose feedback inhibition characteristics are eliminated; and (ii) loss of function mutants in genes encoding enzyme that catabolize the amino acids. Using the metabolic pathway of the essential amino acid lysine, as an example of several model systems that we study, we showed (Fig. 1) that: (i) either the expression of a feedback-insensitive biosynthetic enzyme of this pathway or the loss of function of its catabolic enzyme each caused only a slight increase in lysine level in the cells; (ii) in contrast, combination of these two traits into the same plant caused a huge synergistic in the level of free lysine. These results imply that even though lysine is a minor amino acid in its steady state level, the flux through lysine synthesis and catabolism can become very strong. We are also studying the physiological implications of this strong flux potential through lysine synthesis and catabolism. Our results imply that this metabolic pathway plays multiple physiological functions.

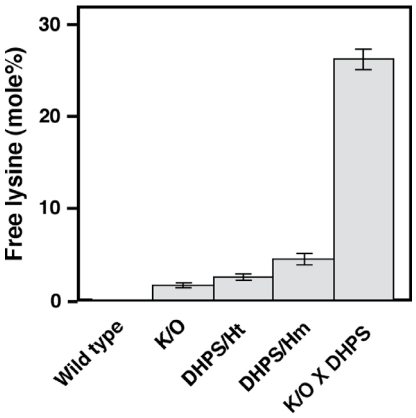

Fig. 1 *Relative free lysine levels in wild type and different transgenic Arabidopsis plants. Relative levels of free lysine are given as mole% of total amino acids. Abbreviations: K/O, a knockout mutant in the LKR/SDH gene of lysine catabolism; DHPS/Ht and DHPS/Hm, transgenic plants expressing a bacterial feedback-insensitive DHPS enzyme of lysine biosynthesis in heterozygous and homozygous states, respectively; K/O × DHPS, the crossed "LKR/SDH knockout" × "DHPS" plants. Bars at the top of the histograms represent the standard error.*

We are also using a bioinformatic approach to elucidate principal transcriptional programs of amino acid metabolism in plants. At the first step, we analyzed the response of metabolic networks associated with the metabolism of 11 out of the 20 protein amino acids in response to abiotic stress conditions, using publicly available microarray results. Our results show that genes encoding catabolic enzymes of amino acids are principally much more sensitive and respond faster to abiotic stresses than genes encoding biosynthetic (allosteric and non-allosteric) enzymes and hence play major regulatory roles in amino acid metabolism upon exposure to these stresses. Yet, the specific spatial and temporal response patterns of genes encoding catabolic and biosynthetic enzymes are distinct for different metabolic pathways in response to different stress conditions. Our results also imply that catabolic enzymes enable rapid and efficient changes of fluxes via different branches of amino acid metabolic pathways upon the exposure to stress conditions, to apparently allow rapid metabolic adjustments. We propose a novel principle module regulating amino acid metabolism in plants (Fig. 2). The major controller of the module is the gene encoding the catabolic enzyme E_t, catalyzing the first catabolic step of the amino acid. Its rapid upregulation in response to external cues stimulates the module by reducing the level of its substrate (S_t), namely the amino acid (Fig. 2; step 1; dotted curved arrow). Reduced level of S_t stimulates the activity of allosteric biosynthetic E_i enzyme by reduction of its feedback inhibitiovn by S_t (Fig. 2; step 2; dashed cured line), stimulating the flux through the entire module. Broken line in Fig. 2 represents all biosynthetic/non-allosteric steps. Hence, the module is regulated by both: (i) a "just-in-time" response of the catabolic genes to external cues when they arrive and (ii) a response of the allosteric biosynthetic enzyme to the levels of the amino acid, "just-in-case" that they are changing in response to changes in the the activity of the catabolic enzyme. Interestingly, many of the metabolic modules are located in branch points of amino acid metabolic networks, allowing rapid shifts in fluxes into different directions through the metabolic networks. Such rapid changes are particularly essential in plants as they are sessile organisms that need to respond rapidly to changing environmental cues.

The pool of the amino acids in plants may also be determined by the extent of their incorporation into proteins (Fig. 2; curved black arrow) and by the extent of protein breakdown (Fig. 2; gray curved arrow). Our proposed module (Fig. 2) may thus also fit to actively growing (non-senescence) tissues of plants grown under favorable (non-stress) conditions in which the catabolic enzymes are generally repressed, but the incorporation of the amino acids into proteins (black curved arrow) may

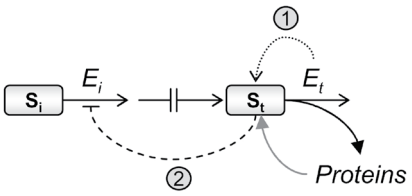

Fig. 2 *Schematic representation of the proposed regulatory metabolic module. The regulatory steps are: The major controller of the module is the gene encoding the catabolic enzyme E_t, catalyzing the first catabolic step of the amino acid. Its rapid upregulation stimulates the module by reducing the level of its substrate (S_t), namely the amino acid (step 1; dotted curved arrow), which then stimulates the activity of the biosynthetic allosteric enzyme (step 2; dashed cured line) and further stimulates the flux through the entire module. Broken line represents all biosynthetic/non-allosteric steps. The pool of the amino acid may also be determined by the extent of its incorporation into proteins (curved black arrow) and by the extent of protein breakdown (gray curved arrow).*

transiently reduce the level of the amino acid (S_t) and as a consequence enhance the flux through the metabolic module by transiently reducing the feedback inhibition on the allosteric enzyme E_i.

In a different project, using the same genotypes described above, we are also studying how do plants respond to changes in fluxes of amino acid metabolic pathways and whether these responses can expose novel regulatory principles of plant metabolism. In this study we use developing and germinating seeds as a model system. Our results expose a number of interesting observations: (i) seed development and germination are associated with distinct metabolic switches in which particular metabolites, such as trehalose, gamma-amino butyric acid (GABA) and glutamine play central regulatory roles; and (ii) the period of seed desiccation, which was previously thought to be mostly associated with loss of water, appears to be highly active in specific metabolic networks that prepare the seed for germination.

Selected publications

Galili G (2002) New insights into the regulation and functional significance of lysine metabolism in plants. Annu. Rev. Plant Physiol. Mol. Biol. 7: 153-156

Zhu X, Galili G (2003) Increased lysine synthesis coupled with a knockout of its catabolism synergistically boosts lysine content and also trans regulates the metabolism of other amino acids in Arabidopsis seeds. Plant Cell 15: 845-853

Zhu X, Galili G (2004) Lysine metabolism is concurrently regulated by synthesis and catabolism in both reproductive and vegetative tissues. Plant Physiol. 135: 129-136

Fait A, Angelovici R, Less A, Ohad I, Urbanczyk-Wochniak E, Fernie AR, Galili G (2006) Arabidopsis Seed Development and Germination Is Associated with Temporally Distinct Metabolic Switches. Plant Physiol. 142: 839-854

Fait A, Fromm H, Walter D, Galili G, Fernie AR (2008) Highway or byway: the metabolic role of the GABA shunt in plants. Trends Plant Sci. 13: 14-19

Less H, Galili G (2008) Principal transcriptional programs regulating plant amino acid metabolism in response to abiotic stresses. Plant Physiol. (in press).

Acknowledgements

The research in our laboratory has been supported by grants from BARD, the United States - Israel Binational Agricultural Research and Development, The Israel Science Foundation (ISF), The US-Israel Binational Science Foundation (BSF), The Germany-Israel Foundation (GIF), the FrameWork Program of the Commission of the European Communities, and by MAGNET.

Gad Galili is an incumbent of the Bronfman Chair in Plant Sciences.

INTERNAL support

Our research on amino acid metabolism over the years was supported in part by MINERVA, The Minna James Heineman Foundation and The Phillip M. Klutznick Fund.

Research in the department of Plant sciences is supported by the The Charles w. and Tillie k. Lubin center for plant biotechnology, The Raymond Burton Center for Plant Genome Research and The Harry and Jeanette Weinberg Center for Plant Molecular Genetics.